Phytoprotection

Characterization of resistance to crenate broomrape (*Orobanche crenata*) in a new small-seeded line of Tunisian faba beans

Caractérisation de la résistance à l'orobanche crénelée *(Orobanche crenata)* dans une nouvelle lignée de féverole à petits grains de Tunisie

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Résumé de l'article

L'orobanche crénelée, *Orobanche crenata*, occasionne des problèmes majeurs à la production de féverole à petits grains dans la région méditerranéenne. Le développement de variétés résistantes demeure le moyen le plus efficace de résoudre ce problème. Les travaux réalisés visaient à estimer le degré de résistance de la lignée sélectionnée XBJ90.03-16-1-1-1-1 à l'orobanche crénelée, en prenant le cultivar Bachaar comme témoin sensible. Dans des champs infestés, les paramètres d'incidence et de sévérité ont été estimés à 95 % et à 4 (sur une échelle de 1 à 9), respectivement, pour 'Bachaar' et seulement à 5 % et 1,5 pour 'XBJ90.03-16-1-1-1-1'. À maturité des cultures, sur la ligne sélectionnée, on observait tout au plus l'émergence d'un individu d'orobanche par plant en moyenne, comparativement à cinq émergences par plant chez le cv. Bachaar. De plus, le rendement grainier de 'XBJ90.03-16-1-1-1-1' a été deux fois plus élevé que celui de 'Bachaar'. En pot, le cv. Bachaar a montré un nombre et un poids sec total d'orobanche par plant plus élevés que ceux de la lignée XBJ90.03-16-1-1-1-1. En chambre racinaire, le taux de germination des graines d'orobanche a été cinq fois inférieur à proximité des racines des plants de la lignée XBJ90.03-16-1-1-1-1 (3 %) qu'à proximité des racines du cv. Bachaar (15 %), ce qui a résulté en un nombre limité de tubercules d'orobanche par plant de 'XBJ90.03-16-1-1-1-1'. Le développement des tubercules a par ailleurs été retardé d'une semaine chez les racines de cette lignée en comparaison au processus infectieux observable sur les racines de 'Bachaar'. Enfin, nos travaux ont souligné quelques caractéristiques des racines de la lignée XBJ90.03-16-1-1-1-1, incluant leur exsudation peu stimulante qui, en combinaison avec un système racinaire profond, favorise la résistance au parasite.

Characterization of resistance to crenate broomrape (*Orobanche crenata*) in a new small-seeded line of Tunisian faba beans

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Orobanche crenata, crenate broomrape, causes major drawbacks in faba bean production in Mediterranean countries. The development of resistant varieties remains the most efficient way to solve this problem. This study was designed to assess the resistance of the breeding line XBJ90.03-16-1-1-1-1 to crenate broomrape, using cv. Bachaar as a susceptible control. Incidence and severity parameters were evaluated in infested fields to values reaching 95% and 4 (on a 1 to 9 scale), respectively, in 'Bachaar', and reaching only 5% and 1.5, in 'XBJ90.03-16-1-1-1-1'. This selected line displayed, at the most, an average of one emerged crenate broomrape individual per plant at crop maturity, whereas 'Bachaar' plants displayed five emerged broomrape individuals under the same conditions. In addition, the seed yield of 'XBJ90.03-16-1-1-1-1' plants was two-fold higher than that of 'Bachaar'. In pot experiments, number and total dry weight of broomrape individuals per plant were significantly higher for 'Bachaar' than for 'XBJ90.03-16-1-1-1-1'. In root chambers, percent germination of broomrape seeds was five-fold lower in the vicinity of 'XBJ90.03-16-1-1-1-1' roots (3%) than close to 'Bachaar' roots (15%). The direct consequence was a limited number of broomrape tubercles per 'XBJ90.03-16-1-1-1-1' plant. Furthermore, tubercle formation on 'XBJ90.03-16-1-1-1-1' roots was delayed by a week compared with the infection process on 'Bachaar' roots. Finally, some features of 'XBJ90.03-16-1-1-1-1' roots were characterized, such as the low amount of exudation of germination stimulant, which, combined with a deep root system, triggers resistance to the parasite.

Keywords: Breeding, germination, *Orobanche crenata*, resistance, *Vicia faba*.

[Caractérisation de la résistance à l'orobanche crénelée *(Orobanche crenata)* dans une nouvelle lignée de féverole à petits grains de Tunisie]

L'orobanche crénelée, *Orobanche crenata*, occasionne des problèmes majeurs à la production de féverole à petits grains dans la région méditerranéenne. Le développement de variétés résistantes demeure le moyen le plus efficace de résoudre ce problème. Les travaux réalisés visaient à estimer le degré de résistance de la lignée sélectionnée XBJ90.03-16-1-1-1-1 à l'orobanche crénelée, en prenant le cultivar Bachaar comme témoin sensible. Dans des champs infestés, les paramètres d'incidence et de sévérité ont été estimés à 95 % et à 4 (sur une échelle de 1 à 9), respectivement, pour 'Bachaar' et seulement à 5 % et 1,5 pour 'XBJ90.03-16-1-1-1-1'. À maturité des cultures, sur la ligne sélectionnée, on observait tout au plus l'émergence d'un individu d'orobanche par plant en moyenne, comparativement à cinq émergences par plant chez le cv. Bachaar. De plus, le rendement grainier de 'XBJ90.03-16-1-1-1-1' a été deux fois plus élevé que celui de 'Bachaar'. En pot, le cv. Bachaar a montré un nombre et un poids sec total d'orobanche par plant plus élevés que ceux de la lignée XBJ90.03-16-1-1-1-1. En chambre racinaire, le taux de germination des graines d'orobanche a été cinq fois inférieur à proximité des racines des plants de la lignée XBJ90.03-16-1-1-1-1 (3 %) qu'à proximité des racines du cv. Bachaar (15 %), ce qui a résulté en un nombre limité de tubercules d'orobanche par plant de 'XBJ90.03-16-1-1-1-1'. Le développement des tubercules a par ailleurs été retardé d'une semaine chez les racines de cette lignée en comparaison au processus infectieux observable sur les racines de 'Bachaar'. Enfin, nos travaux ont souligné quelques caractéristiques des racines de la lignée XBJ90.03-16-1-1-1-1, incluant leur exsudation peu stimulante qui, en combinaison avec un système racinaire profond, favorise la résistance au parasite.

Mots clés : germination, *Orobanche crenata*, résistance, sélection, *Vicia faba*.

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INTRODUCTION

Orobanche species (broomrapes) are parasitic angiosperms whose host range includes members of families such as the Fabaceae, Solanaceae and Apiaceae (Parker and Riches 1993). During the last decade, there has been a continuing increase in infestations of many economically-important crops by some *Orobanche* species (Rubiales *et al.* 2006). Moreover, some invading species, including *O. minor* and *O. ramose* L., are listed as noxious weed in some countries, including the USA and Australia. As a consequence, contaminated lots of crop seeds are quarantined and cannot be exported (Lins *et al*. 2006).

Orobanche seed germination is induced by stimulants in root exudates from host plants. After germination, *Orobanche* seeds produce a specialized organ called a haustorium, which serves as a connection through which water and nutrients are pumped from the host to the parasite. Once attached to the host root, the outer part of the connection zone develops into a tubercle, which gives rise to a spike under favourable conditions (Parker and Riches 1993).

Orobanche crenata Forsk. is one of the most destructive pests in the Mediterranean and Middle East regions. It causes substantial losses and damage in legume crops, especially faba bean. Yield losses can reach 5 to 95% depending on the infestation level and the planting date of faba bean (Mesa-Garcia and Garcia-Torres 1986). Up until now, numerous cultural, mechanical, chemical and biological control strategies have been employed, including catching and trapping crops (Dhanapal *et al*. 1996; Parker and Riches 1993). These strategies remain insufficient, too expensive, or too difficult to employ. At present, the use of resistant varieties is the most economic and environmentally-friendly method of control. In the case of faba bean, this method contributes to a reduced *Orobanche* seed bank in the soil and lower yield losses caused by this plant parasite (Joel *et al*. 2007).

Nevertheless, the mechanisms involved during plant defense in response to *Orobanche* spp. are complex and, except in the case of sunflowers and *O. cumana* Wallr., total resistance to *Orobanche* is rarely observed (Cubero 1991). Scientists from several countries have contributed actively to the production of new varieties that are significantly less susceptible to *O. crenata* and more productive in highly infested fields. Recently, some resistance traits were found in chickpea and pea, giving new hopes of genetic improvement in these crops (Rubiales *et al*. 2003a, b, 2004). In Egypt, breeders have selected several faba bean cultivars that are significantly less susceptible to *Orobanche crenata*, including Giza402 (Nassib *et al*. 1978, 1982), Giza429, Giza674, Giza843, Misr1 and Misr2 (El-Shirbini and Mamdouh 2004). Concomitantly, the variety Baraca was selected in Spain following the same procedures (Cubero *et al.* 1992; Nadal *et al.* 2004). Some breeding lines and populations have been developed by ICARDA (Syria) and distributed to the concerned countries for further evaluation and selection (Khalil *et al.* 2004). In Tunisia, faba bean crops are infested by two *Orobanche* species, *O. crenata* and *O. foetida* Poir. *Orobanche crenata* is restricted to the eastern part of the country while *O. foetida* infests fields of the western and north-central areas (Kharrat and Halila 1994). Since 1989, INRAT (Institut National de la Recherche Agronomique de Tunisie) has carried out a breeding program aimed at creating new faba bean genotypes that are resistant to both *Orobanche* species. In field experiments, the interesting response behaviour of the breeding line XBJ90.03-16-1-1-1-1 (small-seeded line) to *O. foetida* was recently reported (Abbes *et al*. 2007). Its lower susceptibility was due to a decrease in the number of *Orobanche* attachments and a reduction in parasite emergence, but no parasite necrosis was observed on the host roots. The aim of the present study was to assess the resistance level of the breeding line XBJ90.03-16-1-1-1-1 to the parasitic species *O. crenata*, and to describe the mechanisms involved in this resistance. This was achieved through field trials and pot experiments in greenhouses, using hydroponics cultures and root chambers.

MATERIALS AND METHODS

Plant material

Two faba bean genotypes were used: (1) the commercial cv. Bachaar, recently registered in Tunisia under the INRAT/ICARDA collaborative program (Anonymous 2004) and known to be susceptible to *O. crenata*, and (2) the breeding line XBJ90.03-16-1-1-1-1, selected by the INRAT breeding program for its resistance to *O. foetida* (Abbes *et al.* 2007). This line was produced from crosses between a small-seeded pure line of Tunisian faba bean and a breeding line kindly provided by ICARDA (Syria), which carried some resistance traits against *O. crenata* from the Egyptian line Giza 402 (Nassib *et al*. 1978, 1982). Seeds of *O. crenata* were collected in 2001 from a large number of plants over several areas in a faba bean field at Ariana, Tunisia, and stored in the dark at 25°C until used for experimental infestations. Seeds of faba bean and *O. crenata* were surface-sterilized by incubation in 1% calcium hypochlorite for 15 min and washed twice with sterilized water before use.

Field trials

The field trials were carried out in a field naturally infested with *O. crenata* at the INRAT Ariana experimental station, Tunisia (latitude 36°50'N, longitude 10°11'E), during three cropping seasons: 2002-2003, 2004-2005 and 2005-2006. A completely randomized design with two replications was used. Each genotype was sown in rows 4 m long, with 0.5 m inter-row spacing. Twenty-five seeds were sown at equidistant intervals in each row. Crop development was assessed by determining the height and the date on which 50% of the plants had started to flower. All other parameters of yield and infestation estimations were recorded at crop maturity.

Seeds were harvested from each faba bean genotype and grain yields are expressed as g plant^{-1}. In addition, 100 seeds per genotype were weighed. Behaviour and susceptibility of each host genotype were estimated in the infested field through the incidence of parasitism and a severity index. Incidence was estimated using a 0 to 100% scale. On this scale, 0% represented a row in which no *O. crenata* had emerged and 100% represented a row in which all the host plants carried

emerged spikes of *O. crenata*. Severity was estimated using a 1 to 9 scale, in which 1 represented healthy, well-developed plants carrying no emerged parasite (high resistance) and 9 represented dead host plants with extensive parasite emergence (high susceptibility) (Abbes *et al*. 2007).

Emerged parasites were separated manually from host plants and the mean number and dry weight (DW) were determined per host plant. DW was measured after drying fresh samples at 80°C for 48 h. Once the tubercles of *O. crenata* had been removed, the DW of the faba bean roots and shoots was determined.

In addition, three host plants per replicate were dug up at the early pod-setting stage during the last growing season (2005-2006). The roots were gently washed and the developmental stage of *Orobanche* attachments was recorded, using a 1 to 5 scale (1: attachment of haustorium to host root; 2: small tubercles without root development; 3: tubercles with crown roots without shoot formation; 4: underground tubercles with shoot formation; 5: emergence of spikes) (Labrousse *et al*. 2001).

Artificial infestation in pots

The faba bean genotypes were grown in 10 L pots containing a substrate of sterilized soil and river sand (2:1) artificially infested with 10 mg of *O. crenata* seeds per kg (about 2500 *Orobanche* seeds). Plants were grown in a greenhouse at 20 ± 3°C under natural light, since *O. crenata* germinates and develops well within this temperature range. Day length was 11-13 h throughout the experiment, which started in winter and ended in spring (2004-2005). At the flowering and maturity stages, roots of infected plants were gently removed from the substrate, washed with water, and the *Orobanche* attachments were carefully harvested. The number and total DW of attached *O. crenata* per plant were determined. DW was measured after the fresh samples had been dried at 80°C for 48 h.

Characterization of faba bean root architecture

'Bachaar' and 'XBJ90.03-16-1-1-1-1' plants were grown in *Orobanche*-free medium in pots as well as in an aerated Coïc neutrophile nutrient solution (Coïc and Lesaint 1975) in a hydroponics system. This nutrient solution (pH 6.8), containing 3.8 mM KNO_3, 0.3 mM K_2HPO_4, 0.8 mM KH_2PO_4, 0.2 mM $NaNO_3$, 2 mM NH_4NO_3, 3.1 mM $Ca(NO_3)_2$, 0.75 mM $MgSO_4$, 0.2 mM NaCl, 3.7 µM $FeCl_3$, 3.5 µM $ZnSO_4$, 24.3 µM Bo_3H_3, 11.8 µM $MnSO_4$, 1 µM $CuSO_4$ and 0.04 µM $(NH_4)_2MoO_4$, was diluted to half and replaced twice a month. The experiment was conducted in a greenhouse under the environmental conditions described above. Root architecture was analyzed at the pod-setting and flowering stages. Total main root length and length of the portion of the main root carrying secondary roots were measured before the root system was divided into three parts: the top part was 10 cm long, starting from the collar zone, the intermediate part extended from 10 to 20 cm, and the third consisted of the remaining root system below 20 cm. The DW of each root part was obtained after the fresh sample had been dried at 80°C for 48 h.

Root chamber experiments

In order to follow *Orobanche* development on the two host genotypes, root chamber experiments were carried out as described by Kroschel (2001). Seed germination in the vicinity of faba bean roots, parasite attachment on roots as well as the ensuing tubercle development were observed. The chambers consisted of three-sided wood frames (30 cm x 15 cm x 2 cm) covered by two hard transparent plastic covers. The space between the covers was filled with sterilized river sand. White filter paper was placed on the front face between the sand and the plastic cover. Before the frame was covered by the plastic sheet, *O. crenata* seeds were spread uniformly on the filter paper at a density of 25 seeds cm^{-2}. One 10-d-old faba bean seedling, previously grown in sterilized river sand, was transferred into each chamber and put in contact with the parasite seeds. On the front face of the plastic cover, two 2 cm^2 rectangles were drawn at four different depth levels (Fig. 1) in order to estimate the percent germination of *Orobanche* seeds. Then, the root chambers were transferred to a growth chamber (25°C, 12 h photoperiod, 100 µmol m^{-2} s^{-1} photosynthetic active radiations). The chambers were placed side by side into a plastic box in order to shield the roots from light without disturbing shoot development. The plants were watered weekly by moistening the sand with distilled water from the upper and open side of the wood frames.

Total germination (%) was calculated as the mean number of germinated seeds counted at the four depth levels. The total number of tubercles per plant was determined from the whole root system of the host plant. Estimations of percent germination and total number of tubercles were performed weekly for 58 and 90 d after plant transfer (DAT) into the root chambers.

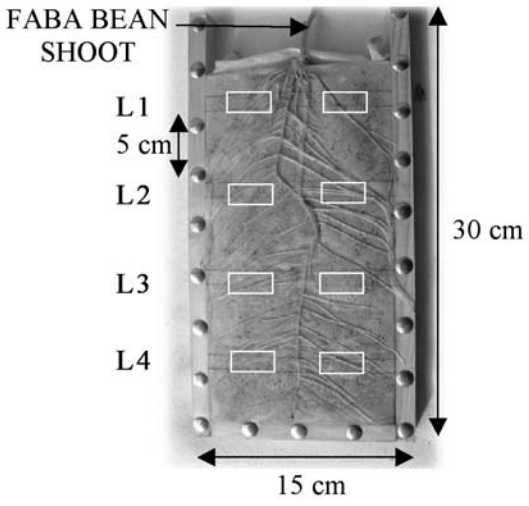

Figure 1. View of the root chamber system used for the analysis of *O. crenata* development on cv. Bachaar and XBJ90.03-16-1-1-1-1 line roots. Percent germination of *O. crenata* seeds was estimated at four depth levels (L) corresponding to the white rectangles, each measuring 2 cm^2. Total number of *O. crenata* tubercles per host plant was estimated from the whole root.

Statistical analysis

Results were analyzed using the SPSS 10.0 software (Windows edition). Mean comparisons were made using Duncan's multiple-range classification test at $P = 0.05$. The statistical model for artificial infestations in pots involved a completely randomized design with five replicates, in which the host genotype was the unique fixed factor. Similarly, the host genotype was also the unique fixed factor for data analyses resulting from the pot and hydroponics experiments in *Orobanche*-free medium (n = five per genotype). The statistical model for the experiment with artificial infestations in root chambers was a randomized design with seven replicates per faba bean genotype, using the host genotype and the root-depth levels as the fixed factors.

RESULTS

Field trials

During the cropping seasons of 2004-2005 and 2005-2006, the incidence of parasitism reached 90-100% for cv. Bachaar (Table 1). Almost all the faba bean plants carried at least one emerged *O. crenata* at crop maturity. An average severity value of 4.5 characterized the impact on host plant development at crop maturity for the susceptible genotype. In contrast, incidence and severity were much lower during both cropping seasons for the breeding line XBJ90.03-16-1-1-1-1, which displayed very few attachments and thus a very low total DW of emerged *O. crenata* per crop plant at maturity. Indeed, parasite emergence was rare and even nil in 2004-2005. Consequently, the selected XBJ90.03-16-1-1-1-1 line displayed a two-fold higher grain yield per plant and larger seeds under infestation than cv. Bachaar. Moreover, flowering was observed significantly earlier for the XBJ90.03-16-1-1-1-1 genotype. The exceptionally dry spring in 2005-2006 led to increased parasitism severity and to a dramatic drop in seed yield for both genotypes. Nevertheless, even under these conditions, the selected XBJ90.03-16-1-1-1-1 line had a much higher seed yield and took fewer days to flower.

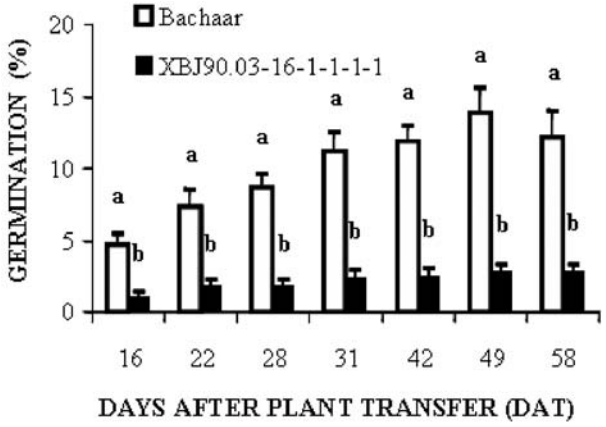

Figure 2. Average germination percentage of *O. crenata* seeds (% of total seeds) in the vicinity of cv. Bachaar and XBJ90.03-16-1-1-1-1 line roots grown under artificial infestation in root chambers. Average percentages were determined weekly during 58 d after plant transfer into root chambers (DAT) by counting the number of germinated seeds at the four depth levels (Fig. 1). Data with the same letter are not significantly different (n = 7; Duncan's test: $P = 0.05$). Data are means ± SE.

Experiments performed during the growing season of 2002-2003 and repeated in 2004-2005 (Table 2) gave more details about the impact of parasitism on host-plant biomass and about the kinetics of the subterranean development of attached *O. crenata*. Development of the susceptible genotype Bachaar was seriously affected by *O. crenata* infection, and in 2002-2003, this genotype displayed a two-fold and five-fold loss of root and above-ground DW, respectively, in comparison with 'XBJ90.03-16-1-1-1-1'. Although the attack by *O. crenata* on 'Bachaar' in 2004-2005 was as strong as during 2002-2003, as indicated by the number and the total DW of attached *Orobanche* per host plant, the Bachaar and XBJ90.03-16-1-1-1-1 genotypes had similar root and shoot biomass DW after infection by the parasite. We suspected that the environmental conditions of the 2004-2005 cropping season were more favourable to 'Bachaar'

Table 1. Indexes of resistance of two faba bean genotypes (cv. Bachaar and the selected XBJ90.03-16-1-1-1-1 line) to broomrape in a naturally *O. crenata*-infested field at Ariana (Tunisia) during two growing seasons (2004-2005 and 2005-2006)

	2004-2005		2005-2006	
Indexes of resistance [a]	Bachaar	XBJ90.03-16-1-1-1-1	Bachaar	XBJ90.03-16-1-1-1-1
Incidence (%)	90.00 ± 10.02 a[b]	0 b	100.00 ± 0.00 a	10.00 ± 10.02 b
Severity (scale 1-9)	3.50 ± 0.50 a	1.00 ± 0.00 b	6.00 ± 0.00 a	2.00 ± 1.00 b
Number of emerged *O. crenata*/host plant	5.24 ± 2.45 a	0 b	5.24 ± 0.29 a	0.03 ± 0.03 b
Emerged *O. crenata* DW/host plant	3.94 ± 1.60 a	0 b	3.40 ± 0.06 a	0.03 ± 0.03 b
Grain yield/host plant (g)	5.56 ± 1.75 b	10.99 ± 0.72 a	0.92 ± 0.22 b	2.17 ± 0.32 a
100 seed weight (g)	46.90 ± 2.50 b	65.15 ± 0.95 a	59.91 ± 2.09 b	72.74 ± 2.26 a
Days to flowering	78.00 ± 0.00 a	74.00 ± 0.00 b	82.00 ± 0.00 a	76.50 ± 0.50 b

[a] Indexes of resistance were estimated at crop maturity.
[b] For each year within a row, values followed by the same letter are not significantly different (Duncan's test: $P = 0.05$). Data are means ± SE.

Table 2. Impact of broomrape attack on biomass at pod-setting stage of two faba bean genotypes (cv. Bachaar and the selected XBJ90.03-16-1-1-1-1 line) in a naturally *O. crenata*-infested field at Ariana (Tunisia) during two growing seasons (2002-2003 and 2004-2005), and indexes of resistance to crenate broomrape

	2002-2003		2004-2005	
	Bachaar	XBJ90.03-16-1-1-1-1	Bachaar	XBJ90.03-16-1-1-1-1
Impact on faba bean biomass				
Root DW (g)	0.84 ± 0.06 b[a]	1.52 ± 0.10 a	2.47 ± 0.23 a	2.19 ± 0.22 a
Shoot DW (g)	4.61 ± 0.18 b	22.73 ± 3.73 a	11.08 ± 0.23 a	11.45 ± 0.36 a
Indexes of resistance [b]				
Total tubercle number/host plant	11.25 ± 2.92 a	1.25 ± 0.25 b	17.17 ± 4.35 a	0 b
Tubercle (stage 2) number/host plant	--	--	7.00 ± 1.69 a	0 b
Tubercle (stage 3) number/host plant	--	--	6.00 ± 1.63 a	0 b
Tubercle (stage 4) number/host plant	--	--	4.17 ± 2.39 a	0 b
Total tubercle DW/host plant (g)	3.26 ± 1.04 a	0.25 ± 0.07 b	2.14 ± 0.99 a	0 b

[a] For each year within a row, values followed by the same letter are not significantly different (Duncan's test: $P = 0.05$). Data are means ± SE. Stages 2, 3 and 4 correspond to small tubercles without root development, growing tubercles with crown roots without shoot formation, and tubercles carrying growing underground shoots, respectively.
[b] Indexes of resistance were measured at the pod-setting stage of faba bean.

than to 'XBJ90.03-16-1-1-1-1' growth. This conclusion was supported by the relatively low value of the severity index (3.5) for cv. Bachaar during 2004-2005, as compared with the index for 2005-2006 (Table 1). Nevertheless, the number and total DW of attached *O. crenata* were significantly higher in the susceptible cultivar than in the breeding line XBJ90.03-16-1-1-1-1 at the pod-setting stage of faba bean, regardless of the environmental conditions (Table 2). Interestingly, no dark necrotic *O. crenata* were observed in 'XBJ90.03-16-1-1-1-1'.

The kinetics of infection by *O. crenata* was studied in the cv. Bachaar in 2004-2005. At the pod-setting stage of the crop, *O. crenata* did not emerge from the soil. Indeed, most of the attached *O. crenata* consisted of young tubercles (stages 2 and 3) from which no stems were yet observable. At crop maturity, only five *Orobanche* spikes had emerged out of 17 *Orobanche* tubercles initially attached to 'Bachaar' roots at the pod-setting stage (Table 1).

Faba bean resistance to *O. crenata* in pot experiments

In order to confirm the comparative susceptibility of the Bachaar and XBJ90.03-16-1-1-1-1 genotypes to *O. crenata*, artificial infestation experiments were carried out in pots in a greenhouse. During these experiments, faba bean plants were dug up at the flowering and maturity stages (Table 3). As expected, the number of attachments and total DW of attached *O. crenata* were much lower for the XBJ90.03-16-1-1-1-1 genotype than for 'Bachaar', regardless of the developmental stage of the host plant. Furthermore, there were more *Orobanche* attachments on 'Bachaar' roots when the host plants had reached maturity.

Architecture of faba bean roots in parasite-free conditions

In pot experiments, measurements of mean root length and the depth of secondary roots at the pod-setting stage indicated that the XBJ90.03-16-1-1-1-1 genotype had a deeper root system than the susceptible cv. Bachaar (Table 4). Nevertheless, we did not observe any significant differences for these parameters during the experiments. On the other hand, for 'Bachaar', in which the upper parts of the root system constituted up to 95% of total root DW, total root biomass (DW) was higher. In contrast, DW of the upper parts of the root system in 'XBJ90.03-16-1-1-1-1' was reduced, but this genotype had a higher total root DW. Additionally, the 'XBJ90.03-16-1-1-1-1' plants had a higher shoot/root ratio than the 'Bachaar' plants.

The tendency of 'XBJ90.03-16-1-1-1-1' plants to display a deeper root system carrying more extended secondary roots was confirmed by plant growth analysis under hydroponics conditions. Indeed, both the depth of secondary roots and total root biomass were significantly higher for the XBJ90.03-16-1-1-1-1 genotype under these conditions. On the other hand, the length of the main root in the Bachaar and XBJ90.03-16-1-1-1-1 genotypes was similar.

Development of *O. crenata* infection process in root chambers

Percent germination of *O. crenata* seeds was recorded weekly in the vicinity of faba bean roots during 56 days after plant transfer into the root chamber (DAT) (Figs. 1 and 2). The parasite seeds started to germinate 16 DAT, regardless of the host genotype. Moreover, percent germination remained lower than 3% in the vicinity of 'XBJ90.03-16-1-1-1-1' roots, thus suggesting a low stimulatory effect of root exudates

Table 3. Indexes of resistance of two faba bean genotypes (cv. Bachaar and the selected XBJ90.03-16-1-1-1-1 line) to crenate broomrape when cultivated in soils artificially infested with *O. crenata*

	At flowering stage		At maturity stage	
Indexes of resistance [a]	Bachaar	XBJ90.03-16-1-1-1-1	Bachaar	XBJ90.03-16-1-1-1-1
Total tubercle number/host plant	1.30 ± 0.25 a[b]	0 b	5.00 ± 0.58 a	2.00 ± 0.67 b
Total tubercle DW (g)/host plant	0.70 ± 0.16 a	0 b	5.17 ± 1.42 a	1.37 ± 0.32 b

[a] Indexes of resistance were measured at both the flowering and maturity stages of faba bean. Inoculation was calibrated to 10 mg of *O. crenata* seeds kg^{-1} of soil in pots.
[b] For each development stage within a row, values followed by the same letter are not significantly different (Duncan's test: $P = 0.05$). Data are means ± SE.

Table 4. Root development of two faba bean genotypes (cv. Bachaar and the selected XBJ90.03-16-1-1-1-1 line) when cultures were carried out in pots and under hydroponics conditions

	Pot experiments		Hydroponics conditions	
Measured parameters [a]	Bachaar	XBJ90.03-16-1-1-1-1	Bachaar	XBJ90.03-16-1-1-1-1
Shoot DW (g)	1.98 ± 0.22 a[b]	2.30 ± 0.20 a	4.37 ± 0.26 a	4.12 ± 0.77 a
Total root DW (g)	1.63 ± 0.23 a	1.22 ± 0.17 a	1.72 ± 0.12 a	1.28 ± 0.20 a
Main root length (cm)	33.50 ± 2.17 a	52.37 ± 8.89 a	32.50 ± 1.50 a	36.00 ± 1.47 a
Depth of secondary roots (cm)	28.50 ± 2.17 a	42.75 ± 7.34 a	27.00 ± 0.70 b	32.00 ± 1.22 a
Root DW (upper part) (g)	1.54 ± 0.21 a	1.06 ± 0.17 a	1.63 ± 0.12 a	1.13 ± 0.19 a
Root DW (intermediate part) (g)	0.06 ± 0.01 a	0.07 ± 0.00 a	0.07 ± 0.01 a	0.10 ± 0.01 a
Root DW (lower part) (g)	0.02 ± 0.01 a	0.09 ± 0.03 a	0.01 ± 0.00 b	0.05 ± 0.00 a

[a] Measurements were done at the pod-setting and flowering stages of faba bean in pot and hydroponics experiments, respectively.
[b] For each culture condition within a row, values followed by the same letter are not significantly different (Duncan's test: $P = 0.05$). Data are means ± SE.

from this genotype. This observation was confirmed by statistical analyses, which showed that the stimulatory capacities of 'XBJ90.03-16-1-1-1-1' root exudates were 5-fold lower than those measured for the 'Bachaar' plants. Although our data suggest that percent germination rose gradually to 14% by 49 DAT in the vicinity of 'Bachaar' roots, this increase was not significant.

The depth levels of 'Bachaar' roots were also taken into account during analyses of the parasite's germination patterns (Figs. 1 and 3A). They revealed that the highest percentages (up to 18%) were recorded at root levels 2, 3 and 4. Germination was significantly lower at root level 1. Moreover, our data suggested that total germination was optimal 49 DAT near 'Bachaar' roots, regardless of the depth. In contrast, percent germination of *O. crenata* seeds did not differ significantly among depth levels near roots of 'XBJ90.03-16-1-1-1-1' (Fig. 3B).

The study of the development of *O. crenata* attachments in root chambers showed that infested 'Bachaar' roots carried on average three tubercles per host plant at 42 DAT (Fig. 4). The development of tubercles attached to 'XBJ90.03-16-1-1-1-1' roots was delayed by 7 d, and the tubercle number per plant remained constant at a very low value (0.3) until 90 DAT. In contrast, 'Bachaar' roots carried about five orobanche tubercles at 90 DAT. This observation clearly separated the response to *O. crenata* by the two genotypes. On the other hand, no necrosis of the parasite was observed before or after attachment to the roots of either genotype.

DISCUSSION

This study describes the behaviour of the Tunisian selected line XBJ90.03-16-1-1-1-1 in response to the parasitic weed *O. crenata* under different culture conditions, including a natural infestation in the field and artificial infestations in pots in a greenhouse and on filter paper in root chambers. These different culture strategies were used to obtain complementary information about the infection process and the mechanisms involved in faba bean resistance to crenate broomrape. In infested fields, the estimation of incidence and severity parameters drawn from the impact of parasitism on plant yield (tolerance index) and the number of emerged *O. crenata* per plant (resistance index), respectively, made differentiation of the tested faba bean genotypes easier. Nevertheless, the estimation of the number of emerged broomrape per host plant was sufficient

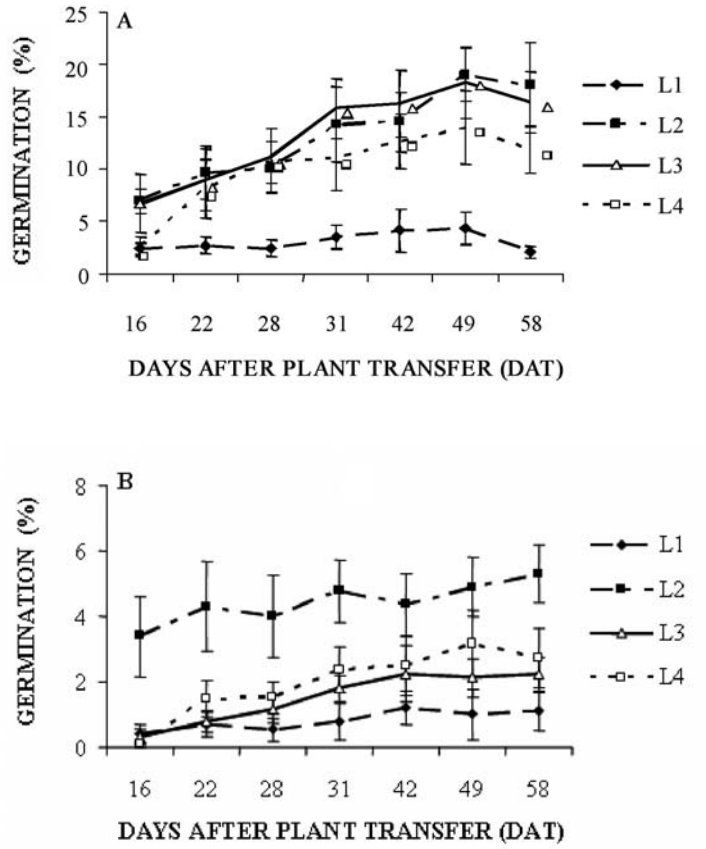

Figure 3. Germination of *O. crenata* seeds (% of total seeds sown) at four different depth levels (L) of the roots of the two faba bean genotypes (A, cv. Bachaar; B, the selected XBJ90.03-16-1-1-1-1 line) grown under artificial infestation in root chambers. Seeds were counted weekly during 58 d after plant transfer into root chambers (DAT). Data are means ± SE (n = 7; Duncan's test: $P = 0.05$).

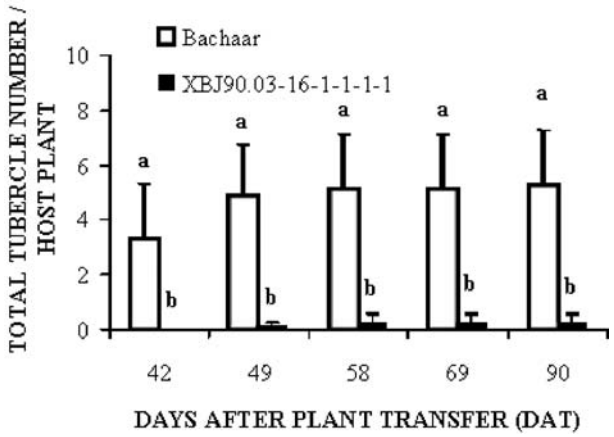

Figure 4. Development of *O. crenata* tubercles on cv. Bachaar and XBJ90.03-16-1-1-1-1 line roots grown under artificial infestation in root chambers. Tubercles were counted weekly during 90 d after plant transfer into root chambers (DAT). Data with the same letter are not significantly different (n = 7; Duncan's test: $P = 0.05$). Data are means ± SE. Regardless of the crop genotype, *O. crenata* seeds germinated starting at 16 DAT. No tubercle was observed before 42 DAT.

in our field trials, as well as during our artificial infestations, to separate the selected XBJ90.03-16-1-1-1-1 line from the susceptible cv. Bachaar. Indeed, the high degree of resistance of the XBJ90.03-16-1-1-1-1 genotype to *O. crenata* only allowed a very low number of *Orobanche* attachments per plant in comparison to the highly parasitized 'Bachaar' plants.

Our results show that the selected XBJ90.03-16-1-1-1-1 line only had partial resistance to *O. crenata* since some *Orobanche* seedlings succeeded in attaching themselves and developing on roots of this genotype. In addition, in the field trials performed over three consecutive cropping seasons, this resistance was expressed under different moisture conditions. Consequently, while seed yields of both genotypes were strongly affected by water stress during the cropping season of 2004-2005, 'XBJ90.03-16-1-1-1-1' plants had higher seed yields than the 'Bachaar' plants, even under dry conditions. These results are consistent with the general observation that host plant tolerance to broomrape drops with decreased water availability (Wegmann 1986). In addition, it has been shown recently that the XBJ90.03-16-1-1-1-1 genotype also displays resistance to *O. foetida* (Abbes *et al*. 2006, 2007). Altogether, these observations confirm the agronomic potential of the XBJ90.03-16-1-1-1-1 line.

Artificial infestations of faba bean cultures in root chambers were used to study the *Orobanche* infection process. This approach demonstrated that the resistance of the XBJ90.03-16-1-1-1-1 genotype was linked to a low stimulatory activity of its root exudates, which failed to induce *Orobanche* seed germination. As a consequence, there were few parasite attachments on 'XBJ90.03-16-1-1-1-1' roots. Although some studies have reported that root exudates collected from resistant and susceptible legume varieties induced similar enhanced germination responses in *O. crenata* seeds (Dörr *et al*. 1994; Goldwasser *et al*. 1997; Khalaf and El-Bastawisy 1989; van Woerden *et al*. 1994), most studies on legume resistance to broomrape concluded that resistance is correlated with low stimulatory activity by root exudates of the host plant (Aalders and Pieters 1986; Abbes *et al*. 2006; Rubiales *et al*. 2003c, 2004 and 2006; Wegmann 1986). Resistance of the parent variety Giza 402 to *O. crenata* was characterized by a low occurrence of germination stimulants in root exudates, few parasite attachments on roots, and necrosis of attached tubercles (Nassib *et al*. 1978). During our study of the response of the breeding line XBJ90.03-16-1-1-1-1 to broomrape, no parasite necrosis was observed before or after attachment to roots. Consequently, these results show that only some of the resistance traits of the resistant parent Giza 402 were transferred to the XBJ90.03-16-1-1-1-1 genotype. The origin of the low stimulatory capacities of 'XBJ90.03-16-1-1-1-1' root exudates was not elucidated in the present study. Most reports available on this topic (Cubero *et al*. 1993; El-Halmouch *et al*. 2006; Serghini *et al*. 2001; Whitney 1978) suggest that the eliciting activity of root exudates depends on the respective concentrations of germination stimulants and germination inhibitors in root exudates. Recently, Mabrouk *et al*. (2007a, b, c) showed that in pea, some nodulating strains of *Rhizobium leguminosarum* were inducing resistance to *O. crenata*. This was mainly achieved in host plant roots by increasing the production of phenolic compounds, which possess some inhibitory activities on *Orobanche* seed germination.

The present study also suggests that the root architecture of 'XBJ90.03-16-1-1-1-1' plants could play a major role in its resistance to *O. crenata*. 'XBJ90.03-16-1-1-1-1' plants have a deep root system with extended secondary roots in the deepest part. In contrast, the upper root parts of 'Bachaar' plants were larger, denser and produced high levels of stimulatory exudates. Zemrag (1999) reported that 80% of *Orobanche* seeds are located in the top 30 cm of the soil profile. This observation is consistent with the fact that the upper roots of 'Bachaar' are responsible for its high susceptibility to broomrape due to its high production of germination stimulants (especially at levels 2 and 3). In contrast, the deeper roots of 'XBJ90.03-16-1-1-1-1' plants mostly avoid the parasite. Furthermore, ter Borg and van Ast (1991) recommended deep ploughing of soils before sowing to help roots of resistant plants to grow more deeply, thus decreasing infection by parasite avoidance.

We observed that 'XBJ90.03-16-1-1-1-1' plants flowered earlier in the field than 'Bachaar' ones. As suggested by Gil *et al*. (1982), Oswald and Ramson (2004) and Rubiales *et al*. (2005), this could contribute to the avoidance of *Orobanche* attack by delaying the life cycle of host plants. In addition, *Orobanche* tubercle development (but not seed germination) was delayed on 'XBJ90.03-16-1-1-1-1' plants. This was observed under artificial infestation in pots as well as in root chambers. This delay could represent a significant advantage of the resistant line over the parasite through a source-sink competition for nutrients between developing pods and growing *Orobanche* tubercles (Manschadi *et al.* 1997). The parasite also emerged later in fields when attached to 'XBJ90.03-16-1-1-1-1' plants (data not shown), confirming a slow development of the infection process on this genotype. Furthermore, resistance of the parent Giza 402 to *O. crenata* might be associated with mechanical barriers (Nassib *et al*. 1984), such as a slight thickening of cell walls in the epidermis, cortex and xylem tissues combined with compact intercellular spaces between the xylem vessels (Khalaf and El-Bastawisy 1989). Further investigations on this topic with the resistant XBJ90.03-16-1-1-1-1 genotype, without ignoring recent histochemical data that have emphasized the major involvement of phenolic accumulation in roots in legume resistance to *O. crenata*, could bring new insights into understanding resistance to *O. crenata* (Perez-de-Luque *et al.* 2004, 2005; Rubiales *et al*. 2006).

In conclusion, we recommend that the resistant breeding line XBJ90.03-16-1-1-1-1 should be used as a major component in integrated control strategies for *O. crenata*. The different traits of 'XBJ90.03-16-1-1-1-1' reported in this study show that this line could be of major interest in breeding programs to develop new resistant lines to *O. crenata*.

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